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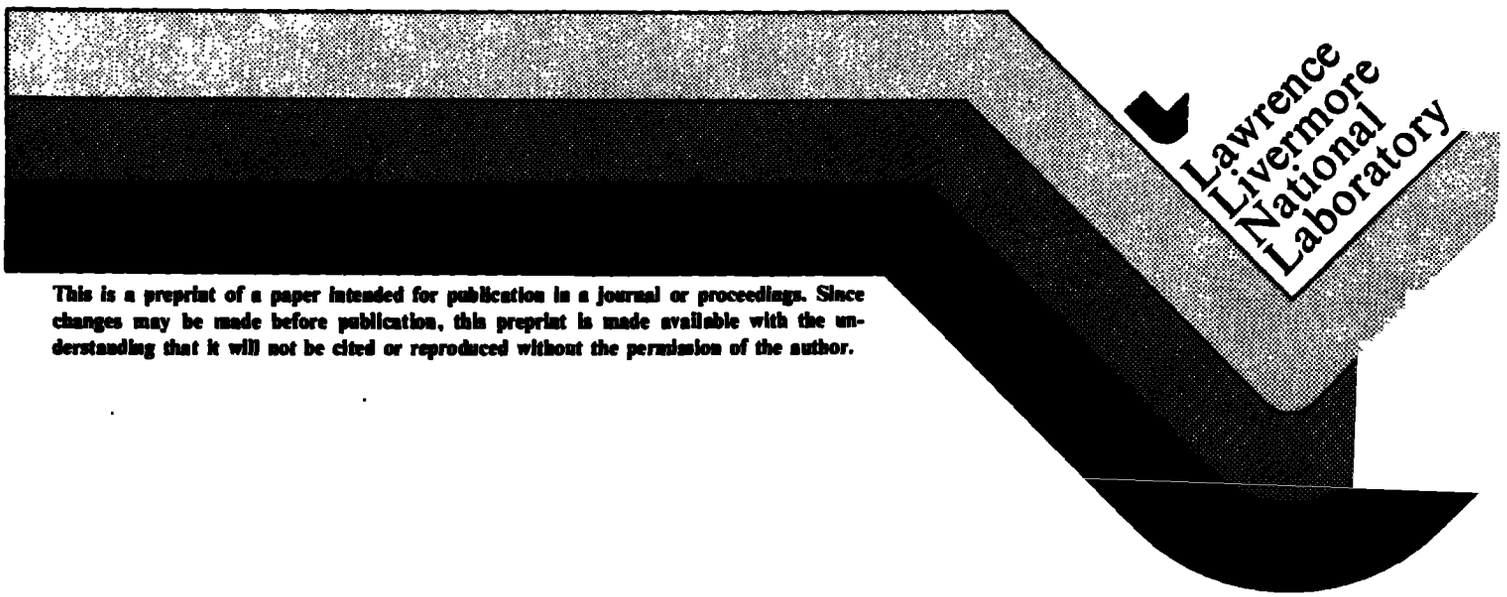

EFFECTS OF RADIATION ON FREQUENCY OF CHROMOSOMAL ABERRATIONS AND SISTER CHROMATID EXCHANGE IN THE BENTHIC WORM *Neanthes arenaceodentata*

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ABSTRACT

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Traditional bioassays are unsuitable for assessing sublethal effects of low levels of radioactivity because mortality and phenotypic responses are not anticipated. We compared the usefulness of chromosomal aberration (CA) and sister chromatid exchange (SCE) induction as measures of low-level radiation effects in a sediment-dwelling marine worm, Neanthes arenaceodentata.

Newly hatched larvae were exposed to two radiation exposure regimes. Groups of 100 larvae were exposed to either x rays delivered at high dose rates (0.7 Gy min^{-1}) or to ^{60}Co gamma rays delivered at low dose rates (4.8×10^{-5} to 1.2×10^{-1} Gy h^{-1}). After irradiation, the larvae were exposed to 3×10^{-5} M bromodeoxyuridine (BrdUrd) for 28 h (x-ray-irradiated larvae) or for 54 h (^{60}Co-irradiated larvae). Slides of larval cells were prepared for observation of CAs and SCEs. Frequencies of CAs were determined in first division cells; frequencies of SCEs were determined in second division cells.

Results from x-ray irradiation indicated that dose-related increases occur in chromosome and chromatid deletions, but an x-ray dose ≥ 2 Gy was required to observe a significant increase. Worm larvae receiving ^{60}Co irradiation showed elevated SCE frequencies; a significant increase in SCE frequency was observed at 0.6 Gy.

Both SCEs and CAs may be useful for measuring effects on genetic material induced by low levels of radiation. However, more detailed studies of both these responses and the factors affecting them are needed before either can be used to quantify the effects of radiation.

1. INTRODUCTION

Since the onset of the nuclear age, radioactive wastes have been disposed on land and in the ocean. In addition, the testing of nuclear weapons has contributed measurable quantities of radionuclides to the ocean. In the United States of America, low-level solid radioactive wastes were disposed in the coastal areas of both the Atlantic and Pacific Oceans (Joseph et al., 1971). Although these practices were discontinued by 1970, little effort was made until recently to determine the subsequent fate and distribution of the radionuclides in these wastes. Information now available indicates that some man-made radionuclides from ocean disposal are present in bottom sediments, but that there is little or no accumulation by organisms in man's food chain (Dyer, 1976; Noshkin et al., 1978).

Increased use of ocean waters for radioactive waste disposal is being considered because land disposal has become difficult and costly. Some opposition to ocean disposal of nuclear waste has been based on fear of irreparable consequences and on the continuing lack of data documenting the effects of oceanic waste disposal. This lack of data can be attributed in part to the absence of appropriate bioassays. Traditional bioassays that use mortality and phenotypic responses as end points are unsuitable for assessing the sublethal effects that may be expected from low-level radioactive waste.

The U.S. Marine Protection, Research, and Sanctuaries Act as amended requires the U.S. Environmental Protection Agency (EPA)

Administrator in reviewing requests for permits to (1) determine that ocean "dumping will not unreasonably degrade or endanger human health, welfare, or amenities, or the marine environment, ecological systems, or economic potentialities" and (2) establish regulations and criteria to implement a permit program (Marine Protection, Research, and Sanctuaries Act, 1972). One possible criterion would utilize a bioassay technique that requires a methodology for detecting the response of marine organisms to low levels of chronic radiation. With such methodology, post-disposal monitoring could verify the assumptions regarding doses to marine organisms and evaluate ultimately the impact of radiation on the organisms.

Deleterious effects of radiation on organisms are well documented (Templeton et al., 1976; U.S. National Academy of Sciences, 1980). Increased cell death and mutations have been related to increased radiation dose. Changes in genetic material include base damage, single-strand breaks, double-strand breaks, hydrogen-bond rupture, and cross-linking between DNA and proteins (Yu, 1976). Some lesions can be detected by examining cells in metaphase for chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs). The numbers of chromosomal aberrations in cells exposed to radiation is much better documented (Blaylock and Trabelka, 1978) than those for SCEs (Kato, 1979).

The CAs are caused by the breakage of chromosomes followed by either the subsequent rejoining of the broken ends to form new combinations or the failure of broken ends to rejoin. They are identified by changes in chromosomal structure that include deletions,

translocations, and rings. It is well established that a substantial part of the changes in DNA induced by ionizing radiation consists of single- and double-strand breaks in the phospho-diester backbone of the DNA molecule. According to Evans (1977), however, all CAs do not result from one, two, or three specific lesions, but are caused by any of a variety of changes in the DNA that lead to helix disruption, helix distortion, or interference with the normal replication process of the cell.

The SCEs represent the interchange of DNA replication products at apparently homologous loci (Latt et al., 1981). This exchange, which does not alter the chromosome morphology, was demonstrated first by autoradiographic techniques using tritiated thymidine (Taylor, 1958). Currently, these exchanges are distinguished by exposing cells to 5-bromodeoxyuridine (BrdUrd) for two rounds of replication and a combined staining with fluorochrome plus Giemsa (FPG) (Perry and Wolff, 1974). Data on SCE frequency in cells exposed to some physical and chemical agents indicate that in mammalian cell systems, SCEs are a sensitive indicator of DNA alterations caused by environmental mutagens and carcinogens.

The effects of ionizing radiation on the frequencies of SCEs have been studied in a number of mammalian cell systems exposed to either β rays (Gibson and Prescott, 1972; Kato, 1974); β plus x rays (Marin and Prescott, 1964; Gatti et al., 1974); x rays (Perry and Evans, 1975; Yu, 1976; Galloway, 1977; Livingston and Dethlefsen, 1979; Morgan and Crossen, 1980; Geard et al., 1981; Nagasawa and Little, 1981; Popescu et al., 1981; Renault et al., 1982; Morgan et al.,

1983); or γ rays (Solomon and Bobrow, 1975; Abramovsky et al., 1978; Littlefield et al., 1979). However, very few data from these experiments are applicable to whole animal, in vivo irradiation. Increases in SCEs were found following in vivo radiation of mice with x rays (Nakanishi and Schneider, 1979). In this study, as in the majority of those using cell systems, dose rates much greater than those expected at ocean-disposal sites were used.

Studies are required to characterize the incidence of CAs and SCEs in organisms that have been irradiated with doses expected at radioactive waste-disposal sites. Nereidae worms are indigenous to marine disposal sites used by the U.S. in the past, and it is expected that they will be present in any future designated areas as well. Because they are benthic and do not migrate, they are well suited to studies of radionuclides and other contaminants that sorb to sediments.

Pesch and Pesch (1980a) proposed that the marine polychaete Neanthes arenaceodentata be used as an in vivo cytogenetic model for marine genetic toxicology. N. arenaceodentata is very suitable for cytogenetic studies because it has 18 large chromosomes. This is in contrast to many invertebrates and fishes that have large numbers of small chromosomes. The effects of ionizing radiation on this species were assessed by quantifying the number of CAs induced by ^{60}Co radiation; at a dose rate of 7.5×10^{-2} Gy h^{-1} and a total dose of 1.8 Gy, an increase in CAs was found (Pesch et al., 1981). Also, a preliminary study on N. arenaceodentata was performed to determine the usefulness of SCE induction as a measure of low-level radiation

effects (Harrison and Rice, 1981). Larvae exposed to ^{60}Co radiation at intermediate doses (0.1 to 0.6 Gy) had SCE frequencies about two times that of the control larvae, but those exposed to high doses (1.6 to 3.0 Gy) had SCE frequencies that approximated those of the control larvae.

Our objective was to assess the feasibility of a cytogenetic approach to detect alterations from radiation in the genetic material of a marine organism. We evaluated the responses to irradiation by using the classical cytogenetic approach of quantifying the frequency of CAs, and by using the more recently developed technique of quantifying the frequency of SCEs.

2. EXPERIMENTAL

2.1. Worm Culture and Handling

N. arenaceodentata were cultured following methods recommended by Reish (1974). Their life cycle is well known and is completed in 3 to 4 mo at 20 to 22°C (Reish, 1957). Mated pairs of adult worms were shipped by Reish in inflated plastic bags containing ~100 ml of seawater. The worms were shipped by U.S. mail in the tubes they had constructed from algae they were fed. Because shipping time seldom exceeded 3 d, worm mortality was low; only a single death occurred during all shipments.

On arrival at Lawrence Livermore National Laboratory, each mated pair of adult worms was placed in a 4-liter glass beaker. The adult worms that produced larvae used in experiments 1 to 6 were cultured using semistatic conditions; the water was aerated continuously and three-fourths of the volume in the beakers was exchanged weekly. Thereafter, adult worms were cultured in 2-liter beakers using flow-through conditions; flow rate through the beaker was 100 ml min^{-1}. Adult worms were maintained for 20 to 30 d in our laboratory before larvae were harvested. The mean culture temperature was 19.4 ± 1.4°C. The adult worms were fed frozen Enteromorpha sp. ad libitum and uneaten food was removed weekly.

2.2. Irradiation

Larvae harvested 1 to 3 d after hatching (3- to 5-setiger stage) were irradiated with x rays generated in a 40-keV x-ray machine and delivered at 0.7 Gy min^{-1}, then examined for CA induction. The doses ranged from 0.08 to 3.8 Gy. Irradiation was conducted in plastic 100- X 20-mm Petri dishes containing 10 ml of seawater. Three thermoluminescent dosimeters (TLDs) were placed in the water along with worms to determine the x-ray dose delivered. A 28-h BrdUrd exposure time was used to obtain the high proportion of first division cells required for CA scoring.

Exposures to ^{60}Co were for 24 h at rates that varied from 4.8×10^{-5} to 1.2×10^{-1} Gy h^{-1}; total doses received varied from 0.01 to 3 Gy. All irradiations were conducted in our low-level

radiation facility equipped with a 4.4 X 10^{10} Bq ^{60}Co source. For each exposure, 50 to 75 worm larvae harvested from 1 to 3 broods were placed in a cylindrical plastic chamber (2.5 cm in diameter) containing 30 ml of filtered seawater. A Plexiglas sheet (5 X 7 X 0.6 cm) was placed in front of each exposure chamber to ensure electron equilibrium. Different dose rates and total doses were obtained by varying the distance between the chamber and the source. Delivered dose was determined from three TLDs placed behind each exposure chamber. A 54-h BrdUrd exposure time was used to obtain the high proportion of second division cells required for SCE scoring.

Two groups of control worms were tested during each experiment. Neither group was irradiated, but one was treated with 5 X 10^{-7} M mitomycin C (MMC), a drug known to increase the frequencies of SCEs, and served as a positive control. Both controls were maintained in the exposure facility during the irradiation of the other groups of worms.

2.3. Cytogenetic Preparation and Scoring of Worm Chromosomes

Immediately following irradiation for experiments 1 to 3, each treatment group of worm larvae was transferred under amber light to 100- X 20-mm plastic culture dishes; a large-bore plastic pipette was used to make the transfer. Each dish contained 30 ml of 3 X 10^{-5} M BrdUrd in filtered seawater. The same procedure was followed in experiments 4 to 30, except 50 ml of seawater in 100-ml glass beakers were used. Nonirradiated control groups were also transferred to the

same concentration of BrdUrd or to BrdUrd plus 5 X 10^{-7} M MMC. The BrdUrd and MMC exposures were carried out in the dark, and colchicine (final concentration of 0.4 mg ml^{-1}) was added to the seawater 4 h before the termination of the BrdUrd exposure. Colchicine is a microtubule disruptor that results in the accumulation of cells in metaphase.

The method of larvae harvest and larval tissue preparation developed by Pesch and Pesch (1980b) was followed. Larvae were transferred to 15-ml conical plastic tubes, the seawater decanted, and 10 ml of 0.075M potassium chloride added. After 12 min, this solution was decanted and the larvae were fixed in three changes of methanol plus acetic acid (3:1). The first fixative change was performed after 5 min and the remaining changes after 15 min each. Fixed larvae (50 to 75) were placed in the depression of a ceramic spot dish and mashed twice with broad-tipped forceps. Next, 1 ml of 60% acetic acid was added, and the mixture was mashed continuously for an additional minute. Two drops of the worm tissues suspended in acetic acid were deposited on the end of a clean microscope slide held at 45°C. Using a disposable plastic pipet, we made 10 to 15 successive transfers of the original drops of tissue suspension to clean areas of the slide. This process resulted in the deposition of cells in a series of rings along the length of the slide. The slides were dried at 45°C before staining. Generally, 4 slides could be made from the macerated tissues of 50 to 75 worm larvae. The slides were prepared within 1 h of the start of fixation to ensure the best spreading of chromosomes.

Differential staining of the sister chromatids was accomplished essentially according to the procedure described by Minkler et al. (1978). Preparations were first stained for 10 min in 5-μg ml^{-1} Hoechst 33258 solution (Aldrich Chemical Company, Inc., Milwaukee, WI) in 0.9% sodium chloride (pH 6). Hoechst-stained slides were rinsed for 5 min in distilled water and air dried for at least 20 min. They were then placed in a shallow, clear plastic tray and covered with 0.067M phosphate buffer (pH 6.8) to a depth of 5 mm. Slides were exposed to ultraviolet light in an M-99 printer (400-W General Electric Mercury Lamp, Colight, Inc., Minneapolis, MN) for 45 min. They then were transferred to 10% Giemsa stain in 0.067M phosphate buffer for 6 to 10 min, air dried, and mounted with coverslips using Permount (Fischer Scientific Company, Fairlawn, NJ). Worm tissue fixation, slide preparation, and staining were all carried out under amber light.

Slides were scored by scanning the entire slide with a Zeiss Universal microscope (Carl Zeiss, Inc., Oberkochen, West Germany) equipped with a 10X objective, 63X objective, 1.25X optovar, and 12.5X oculars. All slides were scored blind, and four people performed the scoring.

The proportions of metaphases identified as first, second, and third divisions after the beginning of BrdUrd exposure were recorded. First-division metaphases were examined for the number of CAs and second-division metaphases for the number of SCEs per metaphase. The number of chromosomes scored was recorded for all

metaphases examined. Data for CAs were recorded only for cells that had 17 or 18 chromosomes that could be scored; data for SCEs were recorded for those that had 15 to 18 chromosomes that could be scored.

3. RESULTS

3.1. Chromosomal Aberrations

Almost all CAs induced by x rays in cells of N. arenaceodentata larvae were chromosomal gaps and chromosome and chromatid deletions (CDs). However, only data on CDs are included because the scoring of gaps is subjective.

The frequency of CDs in cells that received no irradiation was low (Table 1). The mean number of CDs per cell for the 14 control (zero dose) experiments was 0.06 and the individual means from the experiments ranged from 0.00 to 0.22. Differences in the frequencies of CDs reported by different scorers and for different slides were tested for homogeneity of binomial proportions using Cochran's test (Snedecor and Cochran, 1967). There was excellent agreement between scorers when the same slides were scored independently; all differences between scorers were easily accounted for by binomial sampling variablity. There was also good agreement in frequencies reported for different slides within the same experiment, with differences not exceeding those expected from binomial sampling. In contrast to these results, there was significant heterogeneity among the CD rates from different experiments ($p = 0.0016$ based on Cochran's test for homogeneous binomial proportions, Snedecor and Cochran, 1967, p. 240).

Table 1. Chromosome and Chromatid Deletions (CDs) in Cells from *N. arenaceodentata* Nonirradiated Larvae Exposed to BrdUrd for 28 h

Experiment	Number of Scorers[a]	Cells Scored[b]	CDs Scored[b]	CD/Cell
1	3	78	0	0.00
2	3	33	0	0.00
3	3	62	2	0.032
4	3	28	6	0.21
5	1	30	1	0.033
6	3	50	5	0.10
7	3	50	3	0.060
8	2	51	11	0.22
9	1	9	0	0.00
10	3	11	2	0.18
11	2	41	2	0.049
12	1	89	5	0.056
20	1	16	1	0.062
21	3	95	1	0.010

[a] Four scorers were available for the experiments.

[b] For slides scored by more than one person for an individual experiment, the number of cells scored and CDs reported were averaged.

The frequency of CDs was determined also in cells exposed to x rays (Table 2). Weighted (by the number of cells scored) least-squares linear regression was used to determine if there was a linear relationship between dose and CD rate. The regression is highly significant ($F = 76.6$ with 1 and 14 degrees of freedom, $p < 0.01$), and the estimated slope is 0.094 CD per cell per Gray of radiation (standard error = 0.017). However, a prediction based on the least-squares best-fit line is not very reliable because of the extreme heterogeneity of the responses at each dose level. Nevertheless, it appears that doses above 2.0 Gy lead to increased frequencies of CDs.

In the experimental cells scored, the number of CDs per individual cell ranged from one to greater than four. The percentage of the total CDs that occurred singly (one per cell) and multiply (more than one per cell) was compared to the total dose delivered (Table 3). The fraction of the total CDs scored that occurred as more than one CD per cell did not appear to be dose related.

3.2. Sister Chromatid Exchange

The frequency of SCEs in cells from worm larvae exposed to BrdUrd for 54 h, but not to radiation, was determined in 18 different experiments. The SCE frequency per chromosome for the individual experiments ranged from 0.096 to 0.38 (Table 4). These experiments were performed over a 17-mo period and, occasionally, larvae were harvested after varying BrdUrd exposure times (including 54 h). Using

Table 2. Chromosome and Chromatid Deletions (CDs) in Cells from *N. arenaceodentata* Larvae Exposed to X Rays and then BrdUrd for 28 h

Dose (Gy)	Experiment	Number of Scorers[a]	Cells Scored[b]	CDs Scored[b]	CD/Cell
0.08	2	1	41	2	0.049
0.18	5	1	30	2	0.067
0.19	4	1	54	2	0.037
0.22	10	1	29	3	0.10
0.24	2	1	40	8	0.20
0.37	5	1	109	4	0.037
0.45	4	2	22	1	0.045
0.47	2	2	28	2	0.071
0.48	1	2	112	12	0.11
0.88	6	2	66	5	0.076
0.88	12	2	95	7	0.074
1.0	1	2	57	10	0.18
1.6	8	3	12	2	0.17
1.7	12	3	52	7	0.13
2.0	7	3	21	12	0.57
2.2	8	3	61	16	0.26
2.5	3	3	48	36	0.75
2.6	12	2	51	11	0.22

Table 2. (Continued)

Dose (Gy)	Experiment	Number of Scorers[a]	Cells Scored[b]	CDs Scored[b]	CD/Cell
3.6	11	3	34	12	0.35
3.8	12	2	56	14	0.25

[a] Four scorers were available for the experiments.

[b] For slides scored by more than one person for an individual experiment, the number of cells scored and CDs reported were averaged.

Table 3. Percentage of Total Chromosome and Chromatid Deletions (CDs) Occurring as One, Two, Three, or Four CDs per Cell in *N. arenaceodentata* Larvae Exposed to X Rays and to BrdUrd for 28 h

Dose (Gy)	Cells Scored	Number of CDs	Percent of CDs			
			1 CD per Cell	2 CD per Cell	3 CD per Cell	≥ 4 CD per Cell
Control	643	39	63	21	16	0
0.08	41	2	100	0	0	0
0.18 to 0.24	153	15	60	40	0	0
0.37	109	4	100	0	0	0
0.45 to 0.48	164	15	60	13	0	27
0.88 to 1.0	228	22	60	24	0	16
1.6 to 1.7	62	9	57	0	0	43
2.0 to 2.2	82	28	63	15	22	0
2.5 to 2.6	99	47	42	20	38	0
3.6 to 3.8	90	26	56	25	0	19

Table 4. Sister Chromatid Exchanges per Chromosome (SCEs/C) in Cells from N. arenaceodentata Nonirradiated (Control) Larvae Exposed to BrdUrd for 54 h

Experiment	Mean SCEs/C	Standard Deviation	Median SCEs/C	Cells Scored	% HFCs[a]
13	0.096	0.08	0.094	24	0
14	0.20	0.22	0.14	18	16.7
15[b]	0.27	0.22	0.20	21	14.3
16	0.13	0.10	0.13	33	0
17	0.22	0.15	0.18	65	4.6
18	0.30	0.20	0.25	52	26.9
19	0.18	0.19	0.13	40	7.5
20	0.19	0.15	0.18	9	11.1
21	0.13	0.09	0.11	12	0
22	0.17	0.14	0.12	32	3.1
23	0.12	0.10	0.089	52	0
24	0.22	0.25	0.17	21	9.5
25	0.26	0.24	0.17	24	20.8
26	0.15	0.12	0.11	67	1.5
27	0.18	0.13	0.17	73	4.1
28	0.38	0.51	0.17	20	20

Table 4. (Continued)

Experiment	Mean SCEs/C	Standard Deviation	Median SCEs/C	Cells Scored	% HFCs[a]
29	0.18	0.17	0.17	22	9.1
30	0.17	0.32	0.063	18	11.1

[a]Percentage of high-frequency cells (HFCs) (cells with more than 0.44 SCE per chromosome).

[b]Larvae harvested after a 48-h exposure to BrdUrd.

data from larvae harvested at times ranging from 28 to 66 h, we determined that the cell cycle time was about 28 h and had little effect on SCE frequency (Harrison and Rice, 1984).

An analysis of individual results (not shown) revealed that at zero dose, the experiment-to-experiment variability was large compared with that between scorers and slides. The standard deviation from scorer to scorer was 0.033, from slide to slide was 0.037, and from experiment to experiment was 0.056.

Cellular SCEs tended to follow a skewed distribution with medians consistently lower than mean SCEs because of the presence of variable numbers of high-frequency cells (HFCs) (Fig. 1). The HFC is defined by pooling all SCEs from the controls (1059 cells from 18 experiments) and finding, in our case, the 90th percentile of this pooled distribution. In order to have 95% confidence that the estimated percentile will, in fact, contain 90% of the cells, we used the nonparametric procedure described by Walsh (1962) and found the kth-largest SCE value from the pooled sample, where K is given by

$$k = 1059(1.0 - 0.90) + 0.5 - 1.645\sqrt{1059(0.90)(0.10)}$$

where 1059 is our sample size and 0.90 is the percentile expressed as a fraction. (The 1.645 comes from the 95th percentile of the standard normal distribution.) The 90th-largest SCE frequency in our sample is 0.44 SCE per chromosome. Thus, we define an HFC as a cell with more than 0.44 SCE per chromosome.

There is clearly variability in the numbers of HFCs in our control samples. Fortunately, the mean SCEs are reasonably normally distributed with an overall mean (weighted by number of cells scored) of 0.19 SCE per chromosome and a weighted standard deviation of the means of 0.056. Normality of the means was tested using Filliben's order statistic correlation test; a value of 0.988 was obtained, which is well above the 5% critical value of 0.938 (Filliben, 1975).

Worm larvae exposed to different doses of ^{60}Co and then examined for SCE induction after a 54-h exposure to BrdUrd had mean frequencies of SCE that varied with the total dose (Table 5). Analysis of results from individual experiments conducted at comparable doses revealed again that there was significant variability from experiment to experiment. This was caused mainly by significantly high proportions of HFCs in a few of the experiments.

The frequencies of SCEs in cells from larvae receiving 0.6 Gy of ^{60}Co radiation are clearly different from those not receiving radiation (Fig. 2). The frequency distribution is similar to that of larvae exposed to MMC; both groups are characterized by having increased incidences of HFCs.

A least-squares linear regression of mean SCE on radiation dose was performed. When weighted (by number of cells scored) linear regression is performed for all doses, the slope of the best-fit line is not significantly different from zero. This is caused by the low responses at the two highest doses. When these data pairs are omitted, a significant ($p = 0.025$) slope results (0.241 ± 0.008

Table 5. Sister Chromatid Exchanges per Chromosome (SCEs/C) in Cells from N. arenaceodentata Larvae Irradiated with ^{60}Co and Exposed to BrdUrd for 54 h

Dose (Gy)	Experiment	Mean SCEs/C	Standard Deviation	Median SCEs/C	Cells Scored	% HFCs[a]
0.001	22	0.22	0.22	0.17	81	12.3
0.001	23	0.17	0.13	0.13	42	2.4
0.001	24	0.16	0.15	0.11	29	6.3
0.001	25	0.17	0.14	0.12	10	0
0.01	26	0.17	0.14	0.17	152	5.3
0.1	15	0.42	0.34	0.44	25	32
0.1	27	0.17	0.19	0.12	140	5.7
0.3	13	0.55	0.49	0.47	24	50
0.3	14	0.16	0.18	0.11	15	13.3
0.3	15	0.34	0.31	0.19	25	32
0.3	16	0.12	0.11	0.11	49	2
0.3	17	0.23	0.23	0.12	19	10.5
0.3	18	0.30	0.27	0.26	12	25
0.3	19	0.17	0.22	0.13	32	3.1
0.3	21	0.20	0.22	0.12	22	13.6

Table 5. (Continued)

Dose (Gy)	Experiment	Mean SCEs/C	Standard Deviation	Median SCEs/C	Cells Scored	% HFCs[a]
0.6	13	0.41	0.54	0.13	25	24
0.6	14	0.20	0.28	0.06	22	18.2
0.6	15	0.47	0.39	0.33	32	40.6
0.6	17	0.37	0.22	0.39	11	36.4
0.6	18	0.32	0.36	0.22	17	23.5
0.6	19	0.15	0.09	0.12	23	0
0.6	20	0.46	0.60	0.24	15	33.3
0.6	21	0.25	0.22	0.20	6	16.7
1.7	13	0.24	0.21	0.16	10	10
1.7	14	0.15	0.12	0.13	13	0
3.0	13	0.24	0.40	0.17	23	4.3
3.0	14	0.18	0.27	0.17	31	6.5

[a]Percentage of high-frequency cells (HFCs) (cells with more than 0.44 SCE per chromosome).

increase in SCE per chromosome per gray). Again, the best-fit line is not very useful for predicting responses because of the large experiment-to-experiment variability. However, the significant regression indicates that over this range of radiation there is a general rise in SCE frequency with increased radiation dose. A significant increase in SCE frequency occurred at a dose of 0.6 Gy but not at 0.3 Gy (Bonferroni t-test adjusted for seven multiple comparisons; $p = 0.0003$) (Miller, 1966). The absence of a significant difference from control at 0.3 Gy may be a false negative. A difference may have been detected if the sample size were larger.

4. DISCUSSION

The induction of CAs by irradiation has been demonstrated both *in vivo* and *in vitro* in many mammals and in mammalian cell systems (Gebhart, 1981). Although the response to radiation was dose related in mammalian systems, the rate of induction differed with the test system and the cell cycle at the time of irradiation. In the worm larvae, we found that the response was dose related also. The CAs that we quantified, CDs, are one-break aberrations that are considered to be induced linearly with dose. Our studies on the background levels of CA induction indicate that in worm larvae, as in mammalian systems, the incidence of CAs is low. Neither the incidence of true point (intragenic) mutations, which are base-pair changes in DNA, nor gross chromosomal (polygenic) mutations brought about by the breakage of chromosomes is easily quantified at low

radiation doses. This is because very few of these events are found, and it is difficult to get a significant increase over background values unless large numbers of cells are scored.

A reliable method that uses peripheral lymphocytes for determining the exposure of people to ionizing radiation has been developed. Because these cells are long-lived and nondividing, aberrations accumulate. Because few lymphocytes cycle in vivo, aberrations can persist rather than be converted to lethal events as a result of genetic imbalance in cell division. These cells have been shown to act as an integrating dosimeter for ionizing radiation. We suggest that a comparable integrating dosimeter for CAs should be looked for in the worm. Blood cells, reproductive cells, or cells that accumulate at a site of injury or excision in preparation for regeneration may be useful for this purpose.

The induction of SCEs is a sensitive indicator of changes due to mutagenic chemicals-- data available on mammalian cells indicate that the response is more sensitive to chemicals than to ionizing radiation (Gebhart, 1981). Contrastively, our results for worm larvae show that a response significantly different from those of controls was obtained at a lower radiation dose using the SCE rather than the CA frequency as an endpoint. However, the SCE response to doses $\geq$1.7 Gy was not related to dose; the frequency of SCEs appeared to plateau or decline. A plateau in the SCE induction rate in mammals was found for β-radiation dose rates between 0.01 and 0.014 Gy h^{-1} (Gibson and Prescott, 1972; Kato, 1979). More recently, however, Nagasawa and Little (1981) reported a dose-related response in SCE frequencies in the density-inhibited

plateau phase of cultures of mouse 10T-1/2 cells irradiated with x rays. The existence of a plateau or a decline at high doses in SCE induction may limit the usefulness of N. arenaceodentata as an indicator of in vivo environmental exposure to radiation.

The use of SCE frequency in tissues as an integrating dosimeter for chronic exposure of mammalian cells has not yet been established. For those chemical mutagens that are S dependent, DNA repair can potentially remove adducts before the cells enter S phase, resulting in increased variability of response. S dependency means that the substance must be present during replicative DNA synthesis, or the lesions it produces in the chromatin require DNA synthesis to be translated into a structured change. Because the induction of SCEs by radiation is also S dependent, such increased variability can be expected also. We still, however, suggest considering the use of SCE induction in cells as a dosimeter while searching for an integrating system for CAs.

At the Windscale radioactive waste disposal site in the Irish Sea, the maximum possible dose rate is estimated to be about 4.5×10^{-4} Gy h^{-1} (Woodhead, 1980). The lowest dose rate that we used in our study that gave a response statistically greater than that of the control was the induction of SCEs by 2.5×10^{-2} Gy h^{-1} of ^{60}Co (0.6-Gy total dose). Although a small increase in mean SCE frequency was observed at 4.8×10^{-4} Gy h^{-1} of ^{60}Co (0.01-Gy total dose), no significant increase in the number of HFCs was found. The maximum time over which the doses were delivered in our system was 24 h; in natural systems it may be over the life span of the organism.

Increased sensitivity of our bioassay could be obtained by increasing the duration of the exposure to the low dose rates or by reducing the high variability in background SCE frequencies.

Much effort has been directed toward understanding the mechanisms of CA and SCE formation and the relationships between the two types of alterations in genetic material (Gebhart, 1981; Carrano and Moore, 1982). It has been shown that chemicals attach to DNA and produce a variety of lesions that can vary from chemical to chemical (Wolff, 1982). With several chemicals, both SCEs and mutations increase linearly with the dose. Thus, for a given chemical, the ratio of SCEs to mutations is constant over a large dose range (Carrano et al., 1978; 1980). Because this ratio changes for each chemical, it may indicate that of the multitude of lesions produced for a given chemical, some could lead to SCEs and others to mutations, or that the lesions that lead to the induction of mutations are a subset of those that produce SCEs (Carrano and Thompson, 1982). Wolff (1982) states that in any case, the induction of SCEs shows that DNA is being affected and that SCEs are an indicator of damage.

According to Wolff (1982),

> Most geneticists agree that induced mutations are detrimental and, therefore, that any general increase in the mutation rate will also be detrimental. The reasons for this are both empirical and theoretical. For instance, radiation-induced mutations in plants, fruit flies, or any other system that is favorable for genetic analysis usually lead to reduced fitness, i.e., are lethal or semilethal. This makes theoretical sense because all living organisms are the result of eons of evolution and have been selected to fit their particular ecological niche; mutations, which are random changes in the genetic constitution of the organisms, can upset the balance brought about by natural selection. There is, however, a problem in determining exactly how

detrimental the effects of mutations will be and how much damage really will be done, especially after low doses.

Because of the uncertainties in our ability to predict the consequences of changes in chromosomes induced by low levels of irradiation, the presence of these changes should be used currently only to signal potential problems in a population. As more data on radiation effects at low dose levels become available, we may be able to relate changes in chromosomes to those in populations and, in turn, to those in communities.

5. CONCLUSIONS

Nereidae worms, because of their karyotype and life style, are a good cytogenetic model for studying radiation effects on benthic organisms. We used N. arenaceodentata larvae to characterize the rates of induction of CDs and SCEs from exposure to ionizing radiation.

The induction of CDs by x rays delivered at 0.7 Gy min^{-1} was dose related, but doses $\geq$2.0 Gy were required to obtain a response that was significantly different from background. Before this bioassay can be applied to conditions that exist at low-level radioactive waste disposal sites, increased sensitivity is required. We suggest that increased sensitivity of the response in the worm be achieved by identifying a long-lived cell system, similar to that of the lymphocytes in mammals, that can be used as a integrating dosimeter for CAs.

The induction of SCEs by ^{60}Co irradiation appeared to be dose related at total doses $\leq$0.6 Gy but not at higher doses. A response that was significantly different from controls was obtained at 0.6 Gy. The 0.6-Gy dose is lower than the 2.0 Gy required to obtain significant difference for CD frequencies, but higher than that required to monitor most radioactive waste disposal sites. Also, the dose over which SCE induction may be used as an indicator of environmental exposure is limited because of the decline in the dose response at higher doses. Before this bioassay is applied to field conditions, factors producing the decline at higher doses need to be identified. Further, we suggest that the required increased sensitivity of this response be obtained by either decreasing the variability in the response at low dose levels or by identifying a cell system that can be used as an integrating dosimeter for SCEs.

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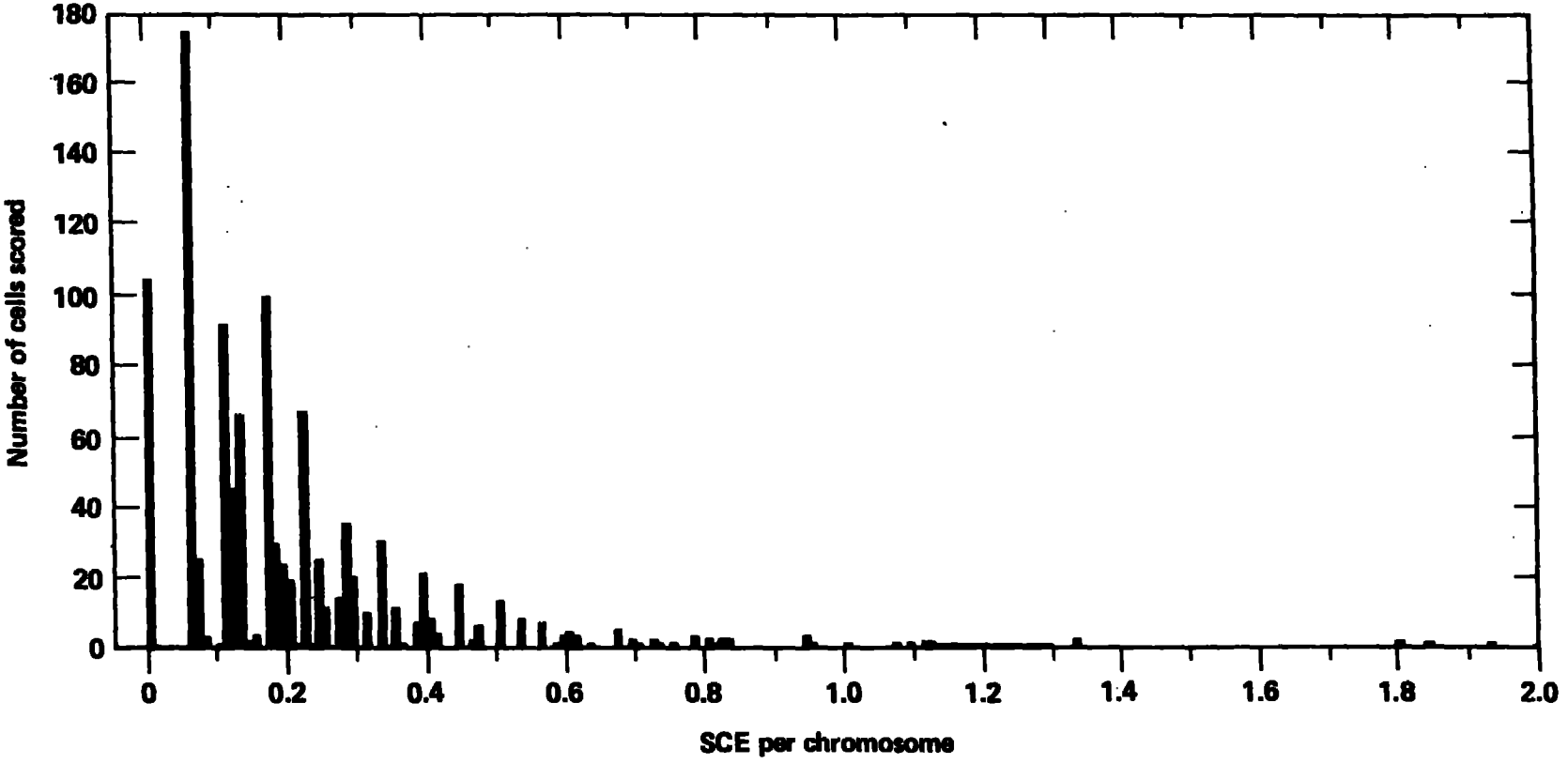
FIGURE LEGENDS

Figure 1. The sister chromatid exchange (SCE) frequency distribution observed in cells of N. arenaceodentata larvae that received no irradiation (controls, 1057 cells from 18 experiments).

Figure 2. The sister chromatid exchange (SCE) frequency distributions observed in cells of N. arenoceodentata larvae that (a) received 0.6-Gy of ^{60}Co radiation, (b) were treated with 5×10^{-7} M mitomycin C (positive control), and (c) received no irradiation (control). Larvae were harvested after a 54-h exposure to BrdUrd. Mitomycin C was present throughout these 54-h; the irradiation preceeded BrdUrd exposure. The total number of cells scored for irradiated, positive-control, and control larvae was 152, 143, and 241, respectively; the mean SCE per chromosome was 0.34, 0.40, and 0.22, respectively.



Harrison
Figure 1

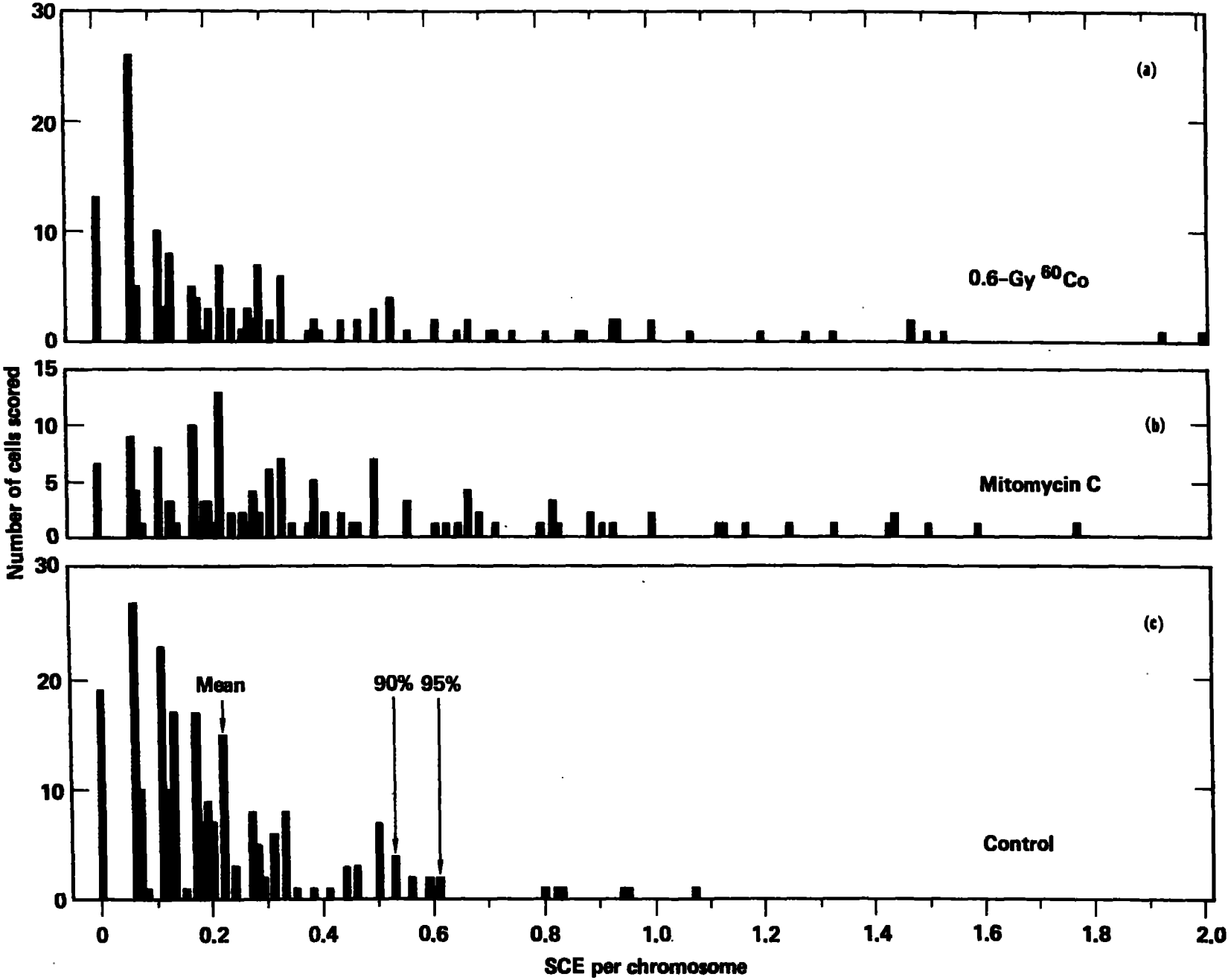


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Figure 2